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RESEARCH LETTER

Differential photoinhibition of bacterial and archaeal ammonia oxidation

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Abstract

Inhibition by light potentially influences the distribution of ammonia oxidizers in aquatic environments and is one explanation for nitrite maxima near the base of the euphotic zone of oceanic waters. Previous studies of photoinhibition have been restricted to bacterial ammonia oxidizers, rather than archaeal ammonia oxidizers, which dominate in marine environments. To compare the photoinhibition of bacterial and archaeal ammonia oxidizers, specific growth rates of two ammonia-oxidizing archaea (*Nitrosopumilus maritimus* and *Nitrosotalea devanaterra*) and bacteria (*Nitrosomonas europaea* and *Nitrosospira multiformis*) were determined at different light intensities under continuous illumination and light/dark cycles. All strains were inhibited by continuous illumination at the highest intensity (500 μE m^{-2} s^{-1}). At lower light intensities, archaeal growth was much more photosensitive than bacterial growth, with greater inhibition at 60 μE m^{-2} s^{-1} than at 15 μE m^{-2} s^{-1}, where bacteria were unaffected. Archaeal ammonia oxidizers were also more sensitive to cycles of 8-h light/16-h darkness at two light intensities (60 and 15 μE m^{-2} s^{-1}) and, unlike bacterial strains, showed no evidence of recovery during dark phases. The findings provide evidence for niche differentiation in aquatic environments and reduce support for photoinhibition as an explanation of nitrite maxima in the ocean.

Introduction

Nitrification is a key process in the cycling of nitrogen in terrestrial and aquatic ecosystems. The first, rate-limiting step of nitrification, the oxidation of ammonia (NH_3) to nitrite (NO_2^-), is carried out by both ammonia-oxidizing bacteria (AOB, Koops & Pommerening-Röser, 2001) and archaea belonging to the recently described thaumarchaea group (AOA, Spang *et al.*, 2010). The first step in ammonia oxidation is catalysed by ammonia monooxygenase, and the subunit A gene (*amoA*) is the most commonly used marker for tracking ammonia oxidizers in environmental samples. Although sharing a common function, bacterial and archaeal *amo* genes are phylogenetically distinct, suggesting different evolution and phenotypic characteristics between AOB and AOA (Nicol & Schleper 2006). AOB were traditionally considered to be responsible for most ammonia oxidation in natural environments, but AOA *amoA* genes are now known to be ubiquitous and to outnumber those of AOB in many environments, including soils (Leininger *et al.*, 2006), oceans (Wuchter *et al.*, 2006), streams (Merbt *et al.*, 2011) and alpine lakes (Auguet *et al.*, 2011). Although AOA and AOB coexist in many ecosystems, differential sensitivities to pH (Nicol *et al.*, 2008), temperature (Tourna *et al.*, 2008) and ammonium concentration (Martens-Habbena *et al.*, 2009; Verhamme *et al.*, 2011) appear to control their relative abundances and activities, suggesting distinct physiological adaptations for each group.

Photoinhibition of ammonia oxidation has been investigated in laboratory cultures of AOB (e.g. Hooper & Terry, 1974, Guerrero & Jones, 1996a, b). Hyman & Arp

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(1992) found that light may completely inhibit nitrite production and *de novo* synthesis of ammonia monooxygenase is required after exposure of cultures to light, leading to suggestions that light may be responsible for the inhibition of nitrification in ocean surface waters (Horrigan *et al.*, 1981), coastal areas (Olson, 1981), estuaries (Horrigan & Springer, 1990) and eutrophic rivers (Lipschultz *et al.*, 1985).

The low availability of laboratory cultures has restricted physiological studies of photoinhibition in AOB and, particularly, AOA. This has prevented assessment of the role of light exposure in niche separation and distribution of AOA and AOB in natural environments. Recent observations of the distribution of archaeal *amoA* genes in stream biofilms exposed to light and dark conditions (Merbt *et al.*, 2011) and along a vertical profile in the Atlantic Ocean (Church *et al.*, 2010) suggest, however, that AOA could also be sensitive to light and that sensitivity of AOA and AOB may differ. The aims of this study were to determine the effects of different light intensities on bacterial and archaeal ammonia oxidation using several laboratory cultures of AOA and AOB and to assess their potential to explain AOB and AOA differential distribution and activity in aquatic ecosystems.

Materials and methods

Strains and culture conditions

Photoinhibition of two AOB (*Nitrosomonas europaea* ATCC19718 and *Nitrosospira multiformis* ATCC25196) and two AOA (*Nitrosopumilus maritimus* and *Nitrosotalea devanaterra*) strains was investigated during growth in batch culture. *Nitrosomonas europaea* and *N. multiformis* were obtained from NCIMB (http://www.ncimb.com/). *Nitrosopumilus maritimus* and *N. devanaterra* were obtained from existing laboratory cultures (Könneke *et al.*, 2005; Lehtovirta-Morley *et al.*, 2011). All strains were grown aerobically in 100-ml quartz flasks containing 50 mL inorganic growth medium. AOB were grown in Skinner & Walker (1961) medium containing 1.78 mM ammonia sulphate, adjusted to pH 8.0 with Na_2CO_3 (5% w/v). *Nitrosopumilus maritimus* was grown in HEPES-buffered, synthetic medium (pH 7.6) (Martens-Habbena *et al.*, 2009), and *N. devanaterra* was cultured in acidic (pH 4.5) freshwater medium as described by Lehtovirta-Morley *et al.* (2011). The media for AOA contained ammonium chloride at concentrations of 1 mM for *N. maritimus* and 0.5 mM for *N. devanaterra*. Media were inoculated with 1% or 10% (v/v) of exponential-phase cultures of AOB or AOA, respectively. Bacterial cultures were sampled (1 mL) at intervals of 8 h for 5 days, and archaeal cultures were sampled daily for 10 days.

Experimental design and sample analysis

Photoinhibition was investigated in controlled temperature chambers maintained at 26 °C and illuminated by compact fluorescent lights (55 W) and clear strip lights (30 W) (International Lamps Ltd, Hertford, UK) emitting light with a wavelength spectrum of 400–680 nm with a maximum intensity at approximately 580 nm. Ammonia-oxidizing activity of the different cultures was measured under continuous illumination at an intensity of either 15, 60 or 500 μE m^{-2} s^{-1} and with diurnal cycles of 8-h light (15 or 60 μE m^{-2} s^{-1}) and 16-h dark conditions. Control cultures were incubated in the dark in the same incubator. Triplicate cultures were grown for all light treatments and controls. Light intensities were selected to reflect conditions prevailing in riparian zones of rivers and lakes, with highest light intensity (500 μE m^{-2} s^{-1}) simulating naturally occurring conditions during a clear summer day in open areas and the lower intensities (60 and 15 μE m^{-2} s^{-1}) simulating conditions in shaded areas.

Ammonia-oxidizing activity was determined by measuring increases in nitrite (NO_2^-) concentration over time for each particular culture and light exposure treatment. Specific growth rate was estimated by linear regression during the linear phase of semi-logarithmic plots of nitrite concentration vs. time, as in previous studies (Powell & Prosser, 1992; Könneke *et al.*, 2005; Lehtovirta-Morley *et al.*, 2011). Estimated specific growth rates in control and illuminated cultures were compared using the Student's *t*-test (two-sample assuming unequal variances).

Results

All AOA and AOB strains grew exponentially during incubation in the dark. Initial increases in nitrite concentration were sometimes non-exponential, because of carryover of nitrite with inocula, but subsequent increases in nitrite concentration were exponential. Typical nitrite production kinetics are exemplified in Fig. 1 for cultures of *N. multiformis* and *N. devanaterra* under continuous light at 60 μE m^{-2} s^{-1} and dark controls. Nitrite production kinetics were analysed prior to limitation by reduction in pH (all strains except *N. devanaterra*) or high nitrite concentration (*N. devanaterra*). Continuous illumination at 60 μE m^{-2} s^{-1} reduced the specific growth rate of *N. multiformis* from 1.05 (±0.07) day^{-1} to 0.62 (±0.01) day^{-1} and completely inhibited that of *N. devanaterra*.

Effects of illumination and associated statistical analysis are summarized in Fig. 2 and Table 1, respectively. AOA were more sensitive to illumination than AOB. Continuous illumination at the lowest light intensity examined (15 μE m^{-2} s^{-1}) did not significantly affect the growth of the AOB, *N. europaea* and *N. multiformis*, but inhibited

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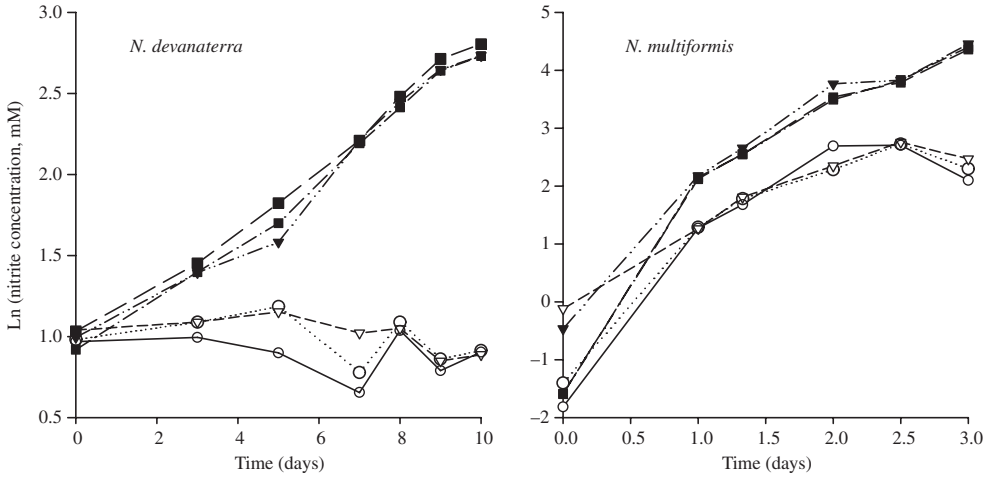

Fig. 1. Semi-logarithmic plots of nitrite concentration vs. time during incubation of triplicate cultures of *Nitrosotalea devanaterra* and *Nitrosospira multiformis* in liquid batch culture in the dark (solid symbols) and under continuous illumination (open symbols) at an intensity of 60 μE m^{-2} s^{-1}.

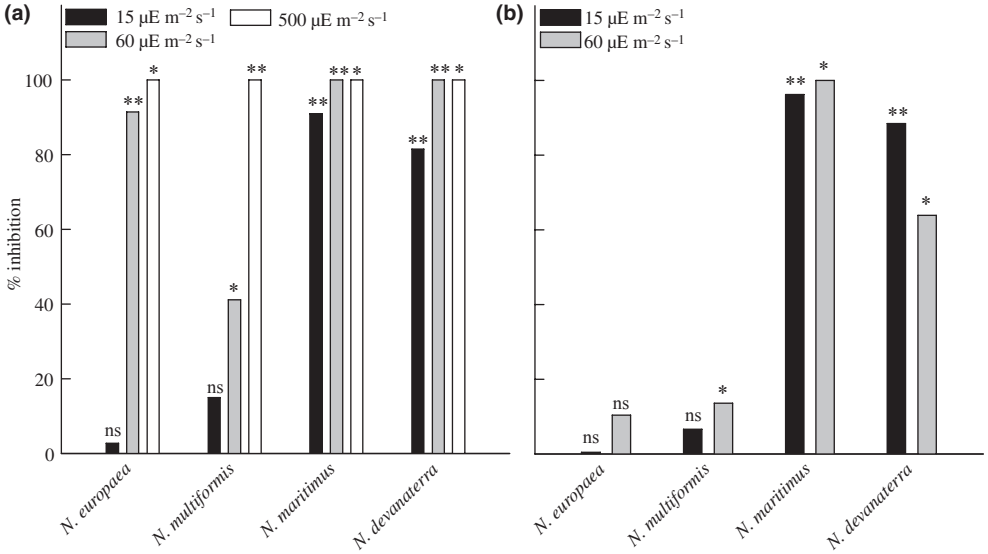

Fig. 2. The reduction in estimated specific growth rate as the percentage of the control, dark-incubated cultures, during incubation of bacterial (*Nitrosomonas europaea* and *Nitrosospira multiformis*) and archaeal (*Nitrosopumilus maritimus* and *Nitrosotalea devanaterra*) ammonia oxidizers under (a) continuous illumination at three intensities (15, 60 and 500 μE m^{-2} s^{-1}) and under (b) 16-h light/8-h dark cycles at 15 and 60 μE m^{-2} s^{-1}. Data are presented as the mean and standard error of triplicate cultures, and significant differences between control and illuminated cultures are represented as ns (no significant difference), $*P < 0.05$ and $**P < 0.001$.

that of the AOA, *N. maritimus* (91% reduced growth rate compared with controls) and *N. devanaterra* (81%) (Fig. 2a, Table 1). Continuous illumination at 60 μE m^{-2} s^{-1} completely inhibited growth of the two studied AOA species, but only partially inhibited growth of AOB strains (Figs 1 and 2, Table 1). The highest light intensity (500 μE m^{-2} s^{-1}) completely inhibited growth of all AOB and AOA strains. Apparent differences in sensitivity to photoinhibition of AOA species were only observed at the lowest light intensity, where *N. devanaterra* was less sensitive than *N. maritimus*. For AOB, *N. europaea* was more sensitive than *N. multiformis*, with respective decreases in specific growth rate of 91% and 41% at 60 μE m^{-2} s^{-1} (Fig. 1, Table 1).

In natural environments, diurnal cycles enable the recovery of ammonia oxidizers from photoinhibition and growth. This was therefore investigated for all strains using 8-h light/16-h dark cycles at the two lowest

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Table 1. Mean estimated specific growth rates of triplicate cultures of bacterial (*Nitrosomonas europaea* and *Nitrosospira multiformis*) and archaeal (*Nitrosopumilus maritimus* and *Nitrosotalea devanaterra*) ammonia oxidizers in liquid batch culture. Cultures were incubated in the dark (control), with continuous illumination or with light/dark cycles

	Light intensity ($\mu E\ m^{-2}\ s^{-1}$)	*N. europaea*		*N. multiformis*		*N. maritimus*		*N. devanaterra*	
		Rate	SE	Rate	SE	Rate	SE	Rate	SE
Continuous illumination	15	1.08	0.007	0.71	0.038	0.019	0.004	0.046	0.003
	Control	1.11	0.017	0.83	0.024	0.21	0.005	0.246	0.008
	P-value	ns		ns		**		**	
	60	0.084	0.007	0.62	0.014	−0.008	0.004	−0.028	0.007
	Control	0.99	0.015	1.05	0.071	0.26	0.001	0.206	0.003
	P-value	**		*		**		**	
	500	ng	ng	ng	ng	−0.017	0.005	−0.018	0.002
	Control	0.62	0.023	1.37	0.010	0.16	0.020	0.081	0.008
	P-value	**		**		*		*	
8:16-h light/dark cycling	15	1.61	0.187	1.45	0.041	0.004	0.002	0.022	0.008
	Control	1.54	0.128	1.56	0.080	0.109	0.003	0.190	0.009
	P-value	ns		ns		**		**	
	60	1.01	0.047	0.88	0.010	−0.004	0.025	0.052	0.007
	Control	1.122	0.011	1.016	0.002	0.211	0.002	0.144	0.004
	P-value	ns		*		*		**	

ng, no growth detected; ns, no significant difference between control and treatment.
*$P < 0.05$;
**$P < 0.001$.

light intensities. At 15 $\mu E\ m^{-2}\ s^{-1}$, AOB were not significantly inhibited, as found under continuous illumination. At 60 $\mu E\ m^{-2}\ s^{-1}$, however, photoinhibition was lower than that under continuous illumination. There was no significant reduction in the specific growth rate of *N. europaea*, demonstrating an ability to recover during periods of darkness, while the growth of *N. multiformis* was reduced by only 14%, compared to 41% under continuous illumination (Fig. 1), suggesting partial recovery. Photoinhibition of *N. maritimus* was not influenced by light cycling, with almost complete inhibition at both light intensities. There was evidence of some recovery of growth of *N. devanaterra* at 60 $\mu E\ m^{-2}\ s^{-1}$, where inhibition was only 63% and surprisingly lower than at 15 $\mu E\ m^{-2}\ s^{-1}$ continuous illumination.

Discussion

Light plays a key role in the nitrogen cycle in aquatic ecosystems, stimulating uptake and excretion of inorganic nitrogen and inhibiting nitrification (Nelson & Conway, 1979; Hooper & Terry, 1973). The detrimental effect of light on ammonia-oxidizing bacteria (AOB) has been known for many years. Hooper & Terry (1973, 1974) demonstrated light inhibition of ammonia oxidation by *N. europaea* suspended cells, with maximum inhibition at short, near-UV wavelength (410 nm). Horrigan & Springer (1990) reported variability in the photosensitivity of ammonia oxidizers such as *Nitrosococcus oceanus* and strain SF-2, isolated from sea-surface films, and Guerrero & Jones (1996a) provided further evidence of species-specific and dose- and wavelength-dependent photoinhibition. Results from the present study support these previous findings.

Photoinhibition appears to operate on the initial step of ammonia oxidation, which is catalysed by ammonia monooxygenase. This step is common to both AOB and AOA, although subsequent metabolism of hydroxylamine, the product of initial ammonia oxidation, has not yet been determined for AOA. Broad similarities in AOA *amoA* gene sequences predict potentially similar AMO structure and therefore similar sensitivities to photoinhibition, while phylogenetic separation of AOA and AOB sequences and other physiological distinctions between archaea and bacteria suggest that levels of photoinhibition may differ and may give rise to niche differentiation, which is supported by our results. The effect of light on AOA has not previously been investigated. This study therefore provides the first evidence of photoinhibition in AOA and significantly greater inhibition of AOA than that of AOB. In addition, the study demonstrates differences in photosensitivity within AOB and AOA. Photoinhibition may therefore contribute to niche differentiation between and within AOA and AOB and may determine their distribution and diversity in light-affected ecosystems.

Our findings influence explanations for several phenomena in aquatic environments. Nitrite often accumulates at the base of the euphotic zone, forming the

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primary nitrite maximum, which is explained by either nitrate reduction to nitrite, by light-limited phytoplankton or by differential photoinhibition of ammonia oxidizers and nitrite oxidizers (Lomas & Lipschultz, 2006). While other environmental factors may drive the distribution of AOA and AOB, the latter hypothesis assumes a key role for photoinhibition of ammonia oxidizers in surface waters, which is relieved with increasing depth, as light intensity decreases. It further assumes that nitrite oxidizers are more photosensitive than ammonia oxidizers, leading to the accumulation of nitrite through greater inhibition of nitrite production and/or slower recovery following photoinhibition. Cultivation-based studies provide contradictory evidence for this hypothesis, indicating that AOB are more photosensitive than nitrite oxidizers (Guerrero & Jones, 1996a), but that they recover more quickly from photoinhibition when subsequently incubated in the dark (Guerrero & Jones, 1996b). However, this model was developed prior to the discovery of the dominance of AOA in marine ecosystems. Greater photoinhibition and slower recovery of AOA, compared with AOB, observed in our study suggest that the difference between photoinhibition of ammonia and nitrite oxidizers is less than previously thought, reducing confidence in this explanation of the nitrite maximum.

The light intensities investigated are similar to those causing *in situ* inhibition of nitrification in previous studies: 100 μE m^{-2} s^{-1} in the eutrophic Delaware River (Lipschultz *et al.*, 1985) and approximately 40–70 μE m^{-2} s^{-1} in a Californian bight (Olson, 1981). In the mixed layer of natural aquatic systems, however, turbidity may promote nitrification both by protecting nitrifiers from photoinhibition and by limiting substrate competition with phytoplankton. Findings also provide a physiological explanation for the higher accumulation of AOA and AOB in river biofilms on the dark side, rather than on the illuminated side of cobbles (Merbt *et al.*, 2011), and the greater abundance of *amoA* genes with decreasing light intensity in the ocean (Church *et al.*, 2010). Despite this evidence of photoinhibition in natural ecosystems, AOA *amoA* abundance is high in regions of high irradiance, such as surface waters of the Mediterranean Sea (Galand *et al.*, 2010) and high mountain lakes (Auguet & Casamayor, 2008; Auguet *et al.*, 2011). This may reflect differences in photosensitivity within AOA, which may also contribute to consistent phylogenetic changes observed in AOA along vertical gradients in the Gulf of Mexico from upper (0–100 m) to deeper layers (450 m) (Beman *et al.*, 2008) and in a deep alpine lake in the Pyrenees (J.C. Auguet, X. Triado-Margarit, N. Nomokonova, L. Camarero & E.O. Casamayor, unpublished data).

Although our findings provide a rationale for future ecological and physiological diversity studies, they were performed with a limited number of strains, of which only one, *N. maritimus*, was isolated from a marine ecosystem. In addition, photoinhibition was investigated in suspended batch culture and may be influenced in natural systems by growth in biofilms and aggregates. Although AOA appear to be more photosensitive, they outnumber AOB in the upper water column (Beman *et al.*, 2008), with high transcriptional activity (Church *et al.*, 2010), and other environmental factors undoubtedly contribute to their relative distributions. Studies of AOB also suggest that photoinhibition depends on wavelength (Hooper & Terry, 1974; Guerrero & Jones, 1996a), which, like intensity, will vary with water depth. Nevertheless, the findings suggest light as an additional factor determining niche differentiation in ammonia oxidizers that may determine their distribution and relative contributions to nitrogen cycling in aquatic ecosystems.

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